ORIGINAL SUBMISSION

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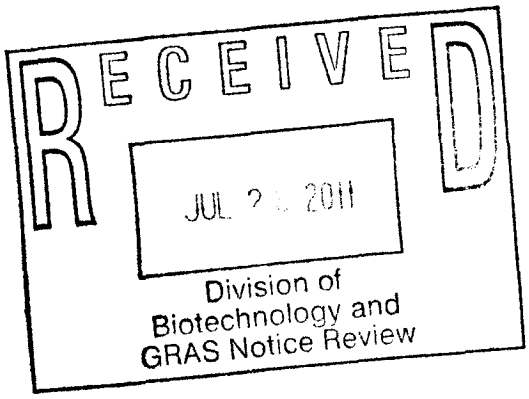

July 25, 2011

Dr. Susan Carlson
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835

Re: GRAS exemption claim for *Chlorella vulgaris* as an ingredient in foods

Dear Dr. Carlson,

This is to notify you that RFI, Inc. claims that the use of the substance described below (*Chlorella vulgaris*) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because RFI has determined such use to be Generally Recognized As Safe (GRAS).

On behalf of RFI, NutraSource (an independent consulting firm) assembled a panel of experts highly qualified by scientific training and experience to evaluate the safety of the intended uses of *Chlorella vulgaris*. The panel included Susan Cho Ph.D., Sechin Cho, M.D., and George Fahey, Jr., Ph.D. Following independent critical evaluation of the available data and information, the panel has determined that the use of *Chlorella vulgaris* (that is manufactured by RFI) described in the enclosed notification is GRAS based on scientific procedures.

After reviewing the available data, the Expert Panel concluded in its July 2011 statement that the intended use of RFI's *Chlorella vulgaris* (to be used as an ingredient in foods, such as granola bars, cereal bars, protein bars and power bars, meal replacements and mixes, sports beverages, energy drinks, energy soft drinks, fruit juices, low calorie fruit and vegetable juice drinks, low fat soy milk, and medical foods), resulting in an estimated 90th percentile daily intake of 1.35 g, is safe and GRAS for the users of *Spirulina platensis*.

This determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

Notifier's name and Address: RFI, Inc.
300 Corporate Drive, suite 14, Blauvelt, NY 10913
Attention: Mr. Paul Altaffer
Phone number: 415-334-7199
Fax number: 415-334-7395
E mail address: Paulo@rfiingredients.com

Name of GRAS substance: *Chlorella vulgaris* (conventional and organic). *Chlorella*, organic *Chlorella*, Sourcestainable® Organic *Chlorella*

Product description:
Chlorella, a class of cyanobacteria, is free-floating filamentous microalgae that is capable of photosynthesis. *Chlorella* has long been a popular functional food worldwide because it is rich in essential nutrients, including high-quality protein, vitamins, minerals, and essential amino acids. *Chlorella* is especially high in protein (approximately 60%) and its amino acid quantity and quality are similar to those of a chicken egg (regarded as a perfect protein) except for methionine and tyrosine. Numerous studies demonstrated physiological benefits of *Chlorella vulgaris,* such as hypoglycemic and hypocholestrolemic effects, immune activation, antioxidant activity, anticarcinogenic activity, antitoxic effects against meta-induced toxicity, dioxin-induced damages, and infection.

Applicable conditions of use of the notified substance:
The proposed use levels of *Chlorella vulgaris* are presented in Table 1.

Table 1. Proposed food application of *Chlorella vulgaris* and maximum levels of use

Proposed food use	Serving size, g	Use level, g/serving	Use level, %
Granola bars, cereal bars, protein bars, and power bars	30	1.5	5
Meal replacement and mixes	240	3.6	1.5
Sports beverages	240	3.6	1.5
Energy drink	40	3.6	9
Energy soft drinks	240	3.6	1.5
Fruit juices, such as lime juice, blackberry juice, grape juice; low calorie fruit and vegetable juice drinks	240	3.6	1.5
Low fat soy milk	240	3.6	1.5
Medical foods	120	12	10

Intended use includes granola bars, cereal bars, protein bars and power bars, meal replacements and mixes, sports beverages, energy drinks, energy soft drinks, fruit juices, low calorie fruit and vegetable juice drinks, low fat soy milk, and medical foods. *Chlorella vulgaris* is not intended for use in meat or poultry-containing products or as a coloring agent.

Assuming that 100% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes from the intended use by users of one or more foods are 13.5 g/d (208 mg/kg BW/d) for the population aged 1 year and above (combining males and females), 15.5 g/d (241 mg/kg BW/d) for males, and 7.5 g/d (139 mg/kg BW/d) for females. After adjustments for market shares (10% of the market share), the 90th percentile intakes by users of one or more foods are 1.35 g/d (20.8 mg/kg BW/d) for the population combining males and females, 1.55 g/d (or 24.1 mg/kg BW/d) for males, and 0.75 g/d (13.9 mg/kg BW/d) for females.

Even if all of the products are used at the maximum levels, exposure estimate levels are much lower than the no-observed-adverse-effect level (NOAEL) values (10,000 mg/kg BW/d) that have been found from toxicity studies in animals and proven safe intake levels of 20-100 g/d which has been found from human clinical trials.

Basis of GRAS determination: Through scientific procedures.

Review and copying statement:
The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of RFI, Inc. or Nutrasource, Inc.

We enclose an original and two copies of this notification for your review. If you have any questions, please contact me.

Sincerely,

(b) (6)

Susan Cho, Ph.D.
Chief Science Officer

Executive Summary

The objective of this Generally Recognized as Safe (GRAS) determination is to summarize the available safety information on *Chlorella vulgaris*, which is used as an ingredient in foods and beverages.

We, the undersigned expert panel members, Susan Cho, Ph.D., Sechin Cho, M.D., and George C. Fahey, Jr., Ph.D., have individually and collectively critically evaluated the materials summarized in the *Chlorella vulgaris* GRAS report. We conclude that *Chlorella vulgaris* is safe and GRAS for its intended use in food. There is broad-based and widely disseminated knowledge concerning the chemistry and health benefits of *Chlorella vulgaris* in both humans and animals.

Pursuant to 21 CFR § 170.30, this GRAS determination for *Chlorella vulgaris* is based upon scientific procedures. There are no indications of significant adverse effects related to *Chlorella vulgaris* in the publicly available literature. In the United States, the safety of *Chlorella vulgaris* has been recognized under DSHEA.

Intended use includes granola bars, cereal bars, protein bars and power bars, meal replacements and mixes, sports beverages, energy drinks, energy soft drinks, fruit juices (grape, blackberry, or lime type), low calorie fruit and vegetable juice drinks, low fat soy milk, and medical foods. *Chlorella vulgaris* is not intended for use in meat- or poultry-containing products or as a coloring agent.

Assuming that 100% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes from the intended use by users of one or more foods are 13.5 g/d (208 mg/kg BW/d) for the population aged 1 year and above (combining males and females), 15.5 g/d (241 mg/kg BW/d) for males and 7.5 g/d (139 mg/kg BW/d) for females (Table 3). After adjustments for market shares, the 90th percentile intakes by users of one or more foods are 1.35 g/d (20.8 mg/kg BW/d) for the population combining males and females, 1.55 g/d (or 24.1 mg/kg BW/d) for males and 0.75 g/d (13.9 mg/kg BW/d) for females.

Even if all of the products are used at the maximum levels, exposure estimate levels are much lower than the no-observed-adverse-effect level (NOAEL) value (10,000 mg/kg BW/d) that has been found from subacute and subchronic toxicity studies in rats. And these levels are much lower than the safe use levels of 20-100 g/d that have been reported in human clinical trials.

The manufacturing process for *Chlorella vulgaris* does not employ any organic solvent treatments. Documentation qualifying a substance as GRAS has been compiled. Such documentation includes technical evidence and common knowledge of safety, as recognized by qualified experts (the Expert Panel). Technical evidence of safety includes the chemical identity of the substance, the method of manufacture, analytical data on composition and specifications, safety data from animal and human clinical studies, and nutritional benefits from animal and human clinical studies. No member of

the Genus Chlorella is known to be toxic. It is our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. A summary of these data is presented herein.

Therefore, the proposed use of *Chlorella vulgaris* is not only safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but it is also GRAS according to Title 21 Code of Federal Regulations (21 CFR) because of this consensus among experts.

Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029

Signature: _____ Date: _____

Sechin Cho, M.D., Professor and Chairman (Retired), Department of Pediatrics, University of Kansas School of Medicine, Wichita, KS 67214

Signature: _____ Date: _____

George C. Fahey, Jr., Ph.D.
Professor Emeritus, University of Illinois, Urbana-Champaign, IL 61801

Signature: _____ Date: _____

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Even if all of the products are used at the maximum levels, exposure estimate levels are much lower than the no-observed-adverse-effect level (NOAEL) value (10,000 mg/kg BW/d) that has been found from subacute and subchronic toxicity studies in rats. And these levels are much lower than the safe use levels of 20-100 g/d that have been reported in human clinical trials.

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Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029
Signature: (b) (6) Date: 7/20/2011

Sechin Cho, M.D., Professor and Chairman (Retired), Department of Pediatrics, University of Kansas School of Medicine, Wichita, KS 67214
Signature: (b) (6) Date: 7/20/2011

George C. Fahey, Jr., Ph.D.
Professor Emeritus, University of Illinois, Urbana-Champaign, IL 61801
Signature: (b) (6) Date: 7/18/11

I. Identity of Substance

A. Common or trade name: *Chlorella*, organic *Chlorella*, *Chlorella vulgaris,* Sourcestainable® Organic *Chlorella*

B. Standards of identity

We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity that is located in Title 21 of the Code of Federal Regulations.

C. Background

Chlorella, a class of cyanobacteria, is free-floating filamentous microalgae that is capable of photosynthesis (Kay, 1991). *Chlorella* has long been a popular functional food worldwide because it is rich in essential nutrients, including high-quality protein, vitamins, minerals, and essential amino acids (Dam et al., 1965; Kay, 1991). *Chlorella* is especially high in protein (approximately 60%) and its amino acid quantity and quality are similar to those of a chicken egg (regarded as a perfect protein) except for methionine and tyrosine (Kang et al., 2004). Numerous studies demonstrated physiological benefits of C*hlorella vulgaris,* such as hypoglycemic and hypocholestrolemic effects, immune activation, antioxidant activity, anticarcinogenic activity, antitoxic effects against meta-induced toxicity, dioxin-induced damages, and infection (An et al., 2008; Bedirli et al., 2009; Estevez et al., 2001; Janczyk et al., 2007; Konish et al., 1985, 1990; Lee et al., 2003, 2008, 2009, 2010; Mizoguchi et al., 2010; Morita et al., 1999; Morris et al., 2007; Nakata et al., 2005; Sano et al., 1988; Sano and Tanaka, 1987; Shibata et al., 2003; Tanaka et al., 2001a, 2001b; Vijayavel et al., 2008; Wang et al., 2010). Over the history of safe use of *Chlorella* by humans, it has been generally recognized as safe (GRAS) for human consumption (Kang et al., 2004).

D. General properties of *Chlorella vulgaris*

Chlorella is a free-flowing, dark blue-green powder with a mild seaweed smell, produced by spray drying the biomass of the cyanobacterium, *Chlorella vulgaris* (Kay, 1991). It is not readily soluble in water or solvents, but it forms a suspension when mixed in water. *Chlorella* species are eukaryotic, unicellular, non-motile freshwater green algae that belong to the Division Chlorophyta (Kay, 1991). *Chlorella* cells have hemicellulosic cell walls and are spherical with a diameter ranging from 2 to 10 µm (Kay, 1991).

E. Manufacturing Process

The RFI's *Chlorella* is produced from the dried biomass of a pure, certified culture obtained from the Academy of Sciences of China. All raw materials used in the growth medium, fermentation process, and production of flour, and all antioxidants used in the

manufacturing process, are suitable food-grade materials and are used in accordance with applicable U.S. federal regulations.

The manufacturing process for RFI's *Chlorella* begins with the selection of *Chlorella* seed meeting specified requirements.

1. This *C. vulgaris* seed is cultured in the lab.
2. The culture is isolated under the microscope and cultured in media using ultra-high purity fertilizer in a number of triangular flasks and glass bottles. These are cultivated at 18-38°C.
3. The cultured *Chlorella* seed then is moved into large ponds that contain food grade fertilizers, water, and carbon dioxide.
4. Conventional *Chlorella* uses the following fertilizers: sodium bicarbonate, potassium chloride, potassium dihydrogen phosphate, and salt. Organic *Chlorella* uses soybean dregs, humic acid, and natural potassium ore powder.
5. Throughout the cultivation process, certain conditions are controlled, such as pH (6.5-7.5), temperature (25-35 °C), and agitation and aeration rates. There are two methods used to judge when *Chlorella* completes growth: a) sensory: deep color of water and *Chlorella* floating on top of ponds, and b) spectrophotometrically (until optical density of a sample reaches 0.8).
6. After harvest, the culture broth is concentrated, optionally washed and/or disrupted (broken cell), and then spray dried.
7. *Chlorella* droplets are sprayed into the chamber from the top of the tower to flash evaporate the water. The temperature is about 180℃. The *Chlorella* broth which is dried into chlorella powder within 5 sec
8. *Chlorella* powder flows into the gather drums and is packaged.

F. Specifications

Table 1 lists specifications of conventional and organic *Chlorella*. *Chlorella* (high-lipid and high-protein) is produced in accordance with current good manufacturing practice (cGMP) and food grade chemical and microbiological specifications have been established for the final products by RFI to ensure consistent, safe products. The chemical and microbiological specifications for high-lipid and high-protein *Chlorella* powders are presented in Table 1.

Table 1a. Specifications of *Chlorella vulgaris* (conventional)

Characteristic	Specification	Specification
Appearance*	Visual	Fine green powder
Sensory	Organoleptic	Mild like sea weed
Particle size	AOAC 973.03 (TQ-106)	100% through 80 mesh
Protein	AOAC 968.06/945.18	≥ 50%
Carotenoids	AOAC 955.10/Paper chromatography	≥ 500 mg/100 g
Chlorophyll	UV spectrophotometry	≥ 1000 mg/100 g
Moisture	TQ-104 (104°C/2h)	≤ 7.0%
Ash	AOAC 923.03	≤ 8.0%
Heavy metals		
Lead	ICP/MS AOAC 993.14	≤ 2.0 ppm
Arsenic	ICP/MS AOAC 993.14	≤ 1.0 ppm
Cadmium	ICP/MS AOAC 993.14	Informative; test annually or every 5 lots (ppm)
Mercury	ICP/MS AOAC 993.14	Informative; test annually or every 5 lots (ppm)
Chromium VI	SW846/7196A (EPA)	Informative; test annually or every 5 lots (ppm)
Microbiology		
Aerobic plate count	AOAC 966.23	≤ 100000 cfu/g
E. coli	USP32, NF27, 2009	Negative/10 g
Salmonella	USP32, NF27, 2009	Negative/10 g
Staphylococcus aureus	USP32, NF27, 2009	Negative/10 g
Coliforms	AOAC 966.24	≤ 10 cfu/g
Yeast/mold	FDA-BAM, 7th ed	≤ 300 cfu/g
Pesticides	FDA 302	Informative; test annually or every 5 lots
Aflatoxins	AOAC 991.31	≤ 20 ppb; test annually or every 5 lots

Table 1b. Specifications of organic *Chlorella vulgaris*

Characteristic	Test Method	Specification
Appearance	Visual	Fine green powder
Protein	AOAC 968.06/945.18	≥ 50%
Carotenoids	AOAC 955.10/ Paper chromatography	≥ 5 mg/g
Chlorophyll	UV spectrophotometry	≥ 25 mg/g
Moisture	TQ-104 (104°C/2h)	≤ 7.0%
Ash	AOAC 923.03	≤ 8.0%
Particle size	AOAC 973.03 (TQ-106)	≥ 95% pass through 80 mesh
Heavy metals		
Lead	ICP/MS AOAC 993.14	≤ 1.0 ppm
Arsenic	ICP/MS AOAC 993.14	≤ 0.5 ppm
Cadmium	ICP/MS AOAC 993.14	Informative (ppm)
Mercury	ICP/MS AOAC 993.14	Informative (ppm)
Chromium VI	SW846/7196A (EPA)	Informative, test annually (ppm)
Microbiology		
Aerobic plate count	AOAC 966.23	≤ 200000 cfu/g
E. coli	USP32, NF27, 2009	Negative/10 g
Salmonella	USP32, NF27, 2009	Negative/10 g
Staphylococcus	USP32, NF27, 2009	Negative/10 g
Listeria	FDA-BAM 8th ed. AOAC OMA 999.06	Negative/ 25 g
Coliforms	AOAC 966.24	≤10 cfu/g
Yeast/mold	FDA-BAM 7th ed.	≤ 500 cfu/g
Aflatoxins	AOAC 991.31	< 20 ppb; test annually

*Breaking cell method: Milling; Broken cell rate tests by microscope method. Cell wall broken ≥ 75%; This is a natural product and there may be color and taste variations from lot to lot due to crop fluctuations from harvest to harvest.
Storage: Store in a cool dry area. Do not freeze. Keep away from strong, direct light.
Shelf life: Two years when properly stored.

All analytical procedures were conducted using standard validated methodologies [i.e., Association of Official Analytical Chemists (AOAC), United States Pharmacopeia (USP), National Formulary (NF), and the U.S. FDA Bacteriological Analytical Manual (FDA-BAM)].

II. Natural occurrence and exposure to *Chlorella vulgaris*

A. Use of *Chlorella* as a foodstuff

Chlorella has been safely consumed as a food ingredient in Mexico and Central Africa for the past four centuries and currently is used as dietary supplements and a novel

food ingredient, especially among Asians, including Asian-Americans (Kang et al., 2004; Kay, 1991).

B. Intended use

Table 2 presents primary applications of *Chlorella vulgaris* that include granola bars, cereal bars, protein bars and power bars, meal replacements and mixes, sports beverages, energy drinks, energy soft drinks, fruit juices (lime, blackberry, or grape juice type), low calorie fruit and vegetable juice drinks, low fat soy milk, and medical foods. *Chlorella vulgaris* is not intended for use in meat or poultry-containing products or as a coloring agent.

Table 2. Intended use of *Chlorella vulgaris*

Proposed food use	Serving size, g	Use level, g/serving	Use level, %
Granola bars, cereal bars, protein bars, and power bars	30	1.5	5
Meal replacements and mixes	240	3.6	1.5
Sports beverages	240	3.6	1.5
Energy drinks	40	3.6	9
Energy soft drinks	240	3.6	1.5
Fruit juices, such as lime juice, blackberry juice, grape juice; low calorie fruit and vegetable juice drinks	240	3.6	1.5
Low fat soy milk	240	3.6	1.5
Medical foods	120	12	10

C. Probable consumption of *Chlorella vulgaris*

Using food intake data reported in the 2005-2008 National Health and Nutrition Examination Survey (NHANES), exposure levels to *Chlorella vulgaris* that will result from the intended uses were estimated (Table 3). The most recent NHANES (2005-2008) compiled by the National Center for Health Statistics and the Nutrition Coordinating Center was used to calculate exposure estimates. The NHANES was conducted between 2005-2008 with non-institutionalized individuals in the U.S. The NHANES provides the most current food consumption data available for the American population. The food and dietary supplement record for each individual includes the gram weight and nutrient data for all foods consumed during the day of the recall. All estimates were generated with USDA sampling weights to adjust for differences in representation of subpopulations. For this study, 1 g is considered equivalent to 1 ml for liquid foods and beverages.

Using food intake data reported in the 2005-2008 NHANES, exposure levels to *Chlorella vulgaris* that will result from the intended uses were estimated. Results of the exposure estimates under the intended use of *Chlorella vulgaris* in foods for the U.S. population ages 1 y and older are presented in Tables 3 and 4.

The first set of estimates is based on the assumption that 100% of the products in each food category will be used at the maximum intake levels under the intended use, although it is far from a realistic situation. The 90th percentile intakes from the intended use by users of one or more foods are 13.5 g/d (208 mg/kg BW/d) for the population combining males and females, 15.5 g/d (241 mg/kg BW/d) for males, and 7.5 g/d (139 mg/kg BW/d) for females (Table 3).

The second set of estimates is based on the market share adjustment which assumes that 10% of the products in each food category will be used at the maximum intake levels under the intended use (Table 4). From a marketing perspective, an assumption that 10% of the product will be used at the maximum levels for each food category is a highly optimistic projection. It is not possible to use all the foods under the intended use. Also, wastage and other losses should be considered. The 90th percentile intakes by users of one or more foods are 1.35 g/d (20.8 mg/kg BW/d) for the population combining males and females, 1.55 g/d (or 24.1 mg/kg BW/d) for males, and 0.75 g/d (13.9 mg/kg BW/d) for females.

These levels are much lower than the NOAEL values (4,000-10,000 mg/kg BW/d) that have been found from subacute and subchronic toxicity studies in rats. Since the *Chlorella vulgaris* level in each food is not listed in the USDA food composition tables and the NHANES databases, the current exposure levels from food sources were not estimated.

Table 3a. Exposure estimate for *Chlorella vulgaris* intakes assuming 100% of products will be used at the maximal levels under the intended use, g/d.

Age, y	All population			Users				
	N	Mean	SE	% of population	Mean	SE	90th	SE
1+ all	17635	0.67	0.042	11.85	5.62	0.21	13.45	0.89
1+ males	8720	1.00	0.075	13.56	7.41	0.35	15.46	0.87
1+ females	8915	0.35	0.020	10.24	3.41	0.11	7.49	0.43
1-3	1521	0.29	0.039	10.24	2.85	0.29	5.61	1.12
4-12	3324	0.58	0.061	14.08	4.15	0.32	9.15	0.46
13-19, all	2851	1.30	0.135	16.79	7.74	0.58	14.64	0.90
13-19, males	1432	1.88	0.249	18.11	10.38	0.73	18.32	3.10
13-19, females	1419	0.71	0.091	15.46	4.60	0.29	10.97	0.68
20+ all	9939	0.61	0.045	10.88	5.64	0.28	13.74	0.90
20+ males	4824	0.97	0.087	12.88	7.55	0.47	16.30	1.36

Table 3b. Exposure estimate for *Chlorella vulgaris* intakes assuming 100% of products will be used at the maximal levels under the intended use, mg/kg BW/d.

Age, y	All population			Users				
	N	Mean	SE	% of population	Mean	SE	90th	SE
1+ all	17635	10.90	0.62	11.85	91.96	3.17	207.62	9.10
1+ males	8720	15.20	1.00	13.56	111.90	4.64	240.89	15.00
1+ females	8915	6.86	0.42	10.24	67.039	3.15	139.22	10.42
1-3	1521	22.52	3.27	10.24	221.44	24.74	448.68	110.5
4-12	3324	18.46	1.76	14.08	131.70	8.80	287.60	16.89
13-19, all	2851	20.28	2.30	16.79	121.15	9.87	226.16	23.44
13-19, males	1432	28.31	4.18	18.11	155.97	14.04	277.52	79.79
13-19, females	1419	12.01	1.47	15.46	78.52	4.49	164.86	17.87
20+ all	9939	7.67	0.54	10.88	70.32	3.10	158.45	13.94
20+ males	4824	11.77	1.00	12.88	91.07	5.47	201.85	19.42

Table 4a. Exposure estimate for *Chlorella vulgaris* intakes after a market share adjustment (assuming that 10% of the products in each food category will be used at the maximum intake levels under the intended use), g/d

Age, y	All population			Users				
	N	Mean	SE	% of population	Mean	SE	90th	SE
1+ all	17635	0.066	0.004	11.85	0.56	0.021	1.35	0.089
1+ males	8720	0.10	0.007	13.56	0.74	0.035	1.55	0.087
1+ females	8915	0.035	0.002	10.24	0.34	0.011	0.75	0.043
1-3	1521	0.029	0.004	10.24	0.28	0.029	0.56	0.111
4-12	3324	0.058	0.006	14.08	0.42	0.032	0.91	0.046
13-19, all	2851	0.130	0.013	16.79	0.77	0.058	1.46	0.090
13-19, males	1432	0.19	0.025	18.11	1.04	0.073	1.83	0.310
13-19, females	1419	0.071	0.009	15.46	0.46	0.029	1.10	0.068
20+ all	9939	0.061	0.004	10.88	0.56	0.028	1.37	0.090
20+ males	4824	0.097	0.009	12.88	0.76	0.048	1.63	0.136

Table 4b. Exposure estimate for *Chlorella vulgaris* intakes after a market share adjustment, mg/kg BW/d

Age, y	All population			Users				
	N	Mean	SE	% of population	Mean	SE	90th	SE
1+ all	17635	1.09	0.062	11.85	9.20	0.32	20.8	0.91
1+ males	8720	1.52	0.100	13.56	11.19	0.46	24.1	1.50
1+ females	8915	0.69	0.042	10.24	6.70	0.31	13.9	1.04
1-3	1521	2.25	0.327	10.24	22.14	2.47	44.9	11.05
4-12	3324	1.85	0.176	14.08	13.17	0.88	28.8	1.69
13-19, all	2851	2.03	0.230	16.79	12.11	0.99	22.6	2.34
13-19, males	1432	2.83	0.418	18.11	15.60	1.40	27.8	7.98
13-19, females	1419	1.20	0.147	15.46	7.85	0.45	16.5	1.79
20+ all	9939	0.77	0.054	10.88	7.03	0.31	15.8	1.39
20+ males	4824	1.18	0.010	12.88	9.11	0.55	20.2	1.94

III. Basis for GRAS determination

Pursuant to 21 CFR § 170.30, *Chlorella* has been determined by RFI to be GRAS on the basis of scientific procedures (U.S. FDA, 2009b). This GRAS determination is based on data generally available in the public domain pertaining to the safety of *Chlorella vulgaris* and other *Chlorella* for use in food and on a consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of *Chlorella* as an ingredient for foods and beverages.

1. Current regulatory status and history of use

A number of *Chlorella* dietary supplement products are available in the U.S. *Chlorella* is included in the United Natural Products Alliance (UNPA) "old" dietary ingredients guidance list, that contains the dietary ingredients that were marketed in the U.S. prior to implementation *of Dietary Supplement Health and Education Act of 1994* (DSHEA, 1994). *Chlorella* is available in capsule, tablet, or powder forms at dosages ranging from 200 to 500 mg/unit (PDRNS, 2001) with recommended dosages up to 10 g/d.

2. Metabolic fate and kinetics

The *Chlorella*-derived proteins, lipids, and carbohydrates are expected to be digested, absorbed, and metabolized through normal physiological processes (PDRNS, 2001), as the macronutrients contained in *Chlorella* are constituents of the normal human diet. Carotenoids in Chlorella are highly available in healthy human volunteers (Shibata and Hayakawa, 2009) as indicated by increases in serum lutein values after consumption of Chlorella: 3 and 6 g of *Chlorella* intake increased serum lutein concentrations by 33 and 66%, respectively. In chicks, the nitrogen absorption coefficient of *Chlorella* was over 80% (similar to that obtained for most of the common feed ingredients) and metabolizable energy was 2.78 kcal/g (comparable to fish meal; Lipstein and Hurwitz, 1980).

3. Animal studies

Table 5 summarizes findings from acute, subacute, subchronic, and reproductive toxicity studies of *Chlorella vulgaris* (Janczyk et al., 2006; Jeong et al., 2009; Krishnaswamy et al., 2000; Lee et al., 2008; Singh et al., 1998). As shown in Table 5, the consumption of *Chlorella vulgaris* at a dose up to 10% in the diet (equivalent to 10,000 mg/kg BW/d), the maximum level tested, had no adverse effects in rats (Lee et al., 2008). In mice, 1% in the diet, which is equivalent to 1,500 mg/kg BW/d, the maximum level tested, did not show any adverse effects (Janczyk et al., 2006). The NOAEL was determined to be 10% in the diet or 10,000 mg/kg BW/d. In addition, no member of the Genus *Chlorella* is known to be toxic.

Table 5. Toxicity studies with *Chlorella vulgaris*

Species	Dose	Study type: Length	Measurements	NOAEL	Ref
Rat (F344; 5/sex/group)	0, 125, 250, 500, 1,000, or 2,000 mg/kg BW/d (deionized water via gavage)	Acute: 1 wk	Food intake, organ wt, histo-pathological changes	2,000 mg/kg BW/d	Krishnas-wamy et al., 2000
Rat (F344; 5/sex/group)	Week 1: 0, 125, 250, 500, 1,000, or 2,000 mg/kg BW/d via gavage; Doses were doubled during the second week to 0, 250, 500, 1,000, 2,000, and 4,000, respectively	Subacute: 2 wk	Food intake, organ wt, urine analysis, histo-pathological changes, liver SGOT and SGPT, and locomotor activity	4,000 mg/kg BW/d	Krishnas-wamy et al., 2000
Rat (Slc: Wistar/ST; 10 M/group)	0, 3, or 5% in diet (equivalent to 0, 3,000, or 5,000 mg/kg BW/d)	Subchronic: 8 wk	Food intake, body wt. gain, liver conc. of AST, ALT, total protein, and albumin, organ wt, blood glucose and insulin, HOMA-IR	5% in diet	Jeong et al., 2009
Rat (Slc: Wistar/ST; 10 M/group)	0, 5, or 10% in normal or high-fat diet (equivalent to 0, 5,000, or 10,000 mg/kg BW/d)	Subchronic: 9 wk	Food intake, body wt. gain, liver conc. of AST, ALT, total protein, and albumin, organ wt, fecal characteristics, and blood lipid profiles	10% in diet	Lee et al., 2008
Mice (Fzt:DU)	0, or 1% in diet	Repro-ductive: 3 generations	Litter size and wt, individual pup wt, survival rate.	1% in diet	Janczyk et al., 2006
Mice (Swiss albino)	0, 100, 300, or 500 mg/kg BW/d via gavage	Repro-ductive: the first 14 d of gestation and lactation	Liver conc. of GST, MDA, and SH and cytochrome b5, and P450 activity of fetuses and neonatals	500 mg/kg BW/d	Singh et al., 1998

SGOT= Serum glutamic oxaloacetate transaminase; SGPT= Serum glutamic pyruvic transaminase; ALT = alanine transaminase; AST = aspartate aminotransferase; M = male; NOAEL = no-observed-adverse-effect level; GST= glutathione S-transferase; MDA=malondialdehyde; SH= sulfhydryl; HOMA-IR=Homeostasis Model of Assessment - Insulin Resistance; 3, 5, or 10% in diet- equivalent to approximately 3,000, 5,000, and 10,000 mg/kg BW/d, respectively (FDA, 1993)

3.1. Acute toxicity study (Krishnaswamy et al., 2000)

Krishnaswamy et al. (2000) investigated acute effects of *Chlorella vulgaris* in rats (up to 2,000 mg/kg BW/d) for 2 wk. Forty male rats weighing between 150-220 g were divided into five different groups to receive the test compound at different dosage concentrations. There were no significant differences in mean body weights, food intakes, or organ weights among treatments and the control groups. The general behavior of all animals was normal. There was no abnormality in heart rate, respiratory rate, or muscle tone. Histopathological examinations showed no dose related responses. There was one preterminal death due to accident while handling and not related to the test compound. Therefore, it was inferred that there were no pathological changes in the organs studied that could be attributed to the test compound administered.

3.2. Subacute toxicity studies of *Chlorella vulgaris* in rats (Krishnaswamy et al., 2000)

Seventy rats (35 males + 35 females) weighing between 200 – 240 g were acclimatized in the experimental room for at least 2 wk prior to study initiation and were randomly assigned to one of 6 groups: a lower dose was fed in the first week followed by doubling of the dose on the following week (week 1: 0, 125, 250, 500, 1,000, or 2,000; deionized water via gavage; week 2: 0, 250, 500, 1,000, 2,000, and 4,000, respectively). As shown in Tables 8-10, there were no significant differences in body weight, food intake, organ weights, or urine analysis results among treatments and the control groups. No abnormality in locomotor activity, touch response, eye orientation, tail pinch, or grip strength was seen in any group of rats. There was no pre-terminal death. There were no abnormalities in the samples of urine tested qualitatively for the presence of albumin, sugar, urobilinogen, or bilirubin, before or after exposure of the test compound at various dose levels.

For clinical chemistry, only liver function tests, including SGOT and SGPT, were conducted in this study. The results did not reveal any significant differences in SGPT or SGOT concentrations among doses. No significant gross changes were noted in the organs of animals from any of the study groups.

Histopathological examination showed no dose-related responses that were non-specific in nature and were not statistically significant. Sporadic, non-dose related histopathological changes were as follows: focal round cell collection in myocardium [0, 2,000, M]; lymphoidal hyperplasia in intestines [0, M; 2,000, F]; focal areas of liver necrosis [0, F; 125, M; 1,000, M]; focal round cell collection in liver [125, F; 250, M; 1,000, M]; round cell collection in glandular stomach [500, F; 1,000, M, F]; peribronchial round cell collection in lungs [125, M]; and varying grades of chronic interstitial pneumonitis in lungs. The authors concluded that the consumption of *C. vulgaris* up to 4,000 mg/kg BW/d caused no treatment associated pre-terminal deaths and no abnormalities in the biochemical and histopathological characteristics of rats.

3.3. Subchronic toxicity studies

Two efficacy studies evaluating hypoglycemic or hyperlipidemic effects of *Chlorella vulgaris* measured hepatic functions, organ weights, BW, and feed intakes in rats. Thus, these studies may be considered as subchronic toxicity studies. The dosages were up to 10% of the diet (equivalent to 10,000 mg/kg BW/d) with durations up to 9 wk.

3.3.1. The study of Jeong et al. (2009)

From an efficacy study investigating hypoglycemic effects, Jeong et al. (2009) reported no adverse effects of *Chlorella vulgaris* on liver function, feed intake, or BW gains in diabetic and normal rats when given experimental diets containing 0, 3, or 5% *Chlorella* (w/w; equivalent to approximately 0, 3,000, or 5,000 mg/kg BW/d, respectively [FDA, 1993]) for 8 wk (Table 5). Liver function measured as plasma aspartate aminotransferase (AST), and alanine aminotransferase (ALT), total protein, and albumin concentrations were within the normal ranges in treatment and control animals, indicating no toxicity of *Chlorella vulgaris*. There were no differences in food intake or BW gain among the groups in either diabetic or normal rats. No abnormalities were observed from the fasting plasma glucagon concentration, the insulin/glucagon ratio, total lipids, triglycerides, total cholesterol concentrations, fasting blood glucose, and plasma insulin as well as Homeostasis Model of Assessment (HOMA) insulin resistance index in both normal and diabetic rats.

3.3.2. The study of Lee et al. (2008)

From an efficacy study investigating hypolipidemic effects, Lee et al. (2008) reported no adverse effects of *Chlorella vulgaris* on liver function, feed intake or BW gain in rats fed normal or high fat diets containing 0, 5, or 10% (w/w; equivalent to approximately 0, 5,000, and 10,000 mg/kg BW/d, respectively [FDA, 1993]) *Chlorella* for 9 wk (Table 5). In both normal and high fat diet groups, liver weight was higher in the control group than in the 5 and 10% *Chlorella* groups ($P<0.05$). However, these values were within the normal range. Kidney weight was significantly affected by dietary fat level. The *Chlorella* level did not affect kidney and spleen weights. Epididymal and perirenal fat pad weights tended to be lower in *Chlorella* intake groups without statistical significance. Brown fat pad weight was lower in the high fat 10% *Chlorella* group compared with the high fat control group ($p<0.05$). Epididymal fat pad weight was significantly affected by dietary fat concentration, and brown fat pad weight was significantly affected by dietary *Chlorella* concentration. The decrease in liver weight was not accompanied by any significant changes in serum AST or ALT activities or total protein and albumin concentrations, indicating no hepatic toxicity of *Chlorella vulgaris*. The changes in liver weight and fat pad weight may be due to the fact that the

consumption of *Chlorella* also significantly increased the fecal excretion of total lipids (by 30-42%), triglycerides (by 149-664%), and total cholesterol (by 217-368%), as well as fecal wet and dry weights (by 23-60%).

In high fat 10% *Chlorella* diet groups, liver total lipids and total cholesterol concentrations were significantly decreased by 71 and 70% compared to the high fat control group. Rats treated with 5 and 10% *Chlorella* had significantly lower liver triglyceride concentration by 72 and 66%, respectively, than that of the high fat control group. The liver total lipids, triglyceride, and total cholesterol concentrations were not significantly affected in normal diet groups. The authors suggested that *Chlorella vulgaris* was effective for prevention of dyslipidemia which may be due to modulation of lipid metabolism.

3.4. Reproductive and teratogenic toxicity studies of *Chlorella vulgaris*

3.4.1. The study of Janczyk et al., 2006

The absence of adverse reproductive effects from consumption of 1.0% *Chlorella vulgaris* (equivalent to approximately 1,560 mg/kg BW/d [FDA, 1993]) was demonstrated in a three-generation reproduction study with Fzt:DU mice (Janczyk et al., 2006). In this study, females from F0 were fed a commercial chow (control diet or *Chlorella*- supplemented chow) starting from d 21 of life (weaning). They were mated randomly on the 63rd day of life and all gave birth to pups. Litters were weighed, counted, and then standardized (4 males and 5 females per litter). Pups were weighed and counted on d 10 and 21. After weaning, 2 females and 2 males (F1) from each litter were kept. Females were mated on d 63 of life. On the 18th day of pregnancy, 57 and 59 (control and algae group) were sacrificed; 51 and 53, respectively, gave birth to pups. Live, dead, absorbed fetuses, and corpora lutei were counted, and live fetuses were weighed. Born pups were counted and weighed and kept with dams without standardization, their number and weight recorded on d 10 and 21. Two females and 2 males (F2) were weaned and kept, and the procedure outlined above was repeated. As shown in Tables 18-22, no differences in numbers of fetuses, corpora lutei, or born pups were noted between treatment groups or between generations. Litters from the algae group were slightly heavier at weaning (P=NS). Females and males from the algae group also developed slightly better than the ones from the control group. This tendency was noted for all generations.

3.4.2 The study of Singh et al. (1998)

Singh et al. (1998) investigated the effects of *C. vulgaris* on the activity of fetal and neonatal hepatic drug metabolizing enzymes and markers of lipid peroxidation in pregnant and lactating Swiss albino mice for the first 14 d of gestation and lactation. *C. vulgaris* was administered by gavage at levels of 0, 100, 300, or 500 mg/kg BW/d. The livers were excised and assayed for glutathione S-transferase (GST), cytochrome b5, and cytochrome P450 activity and malondialdehyde (MDA) and sulfhydryl (SH) levels.

Significantly increased levels of SH and GST were observed in fetal and neonatal livers at *C. vulgaris* doses of 300 or 500 mg/kg BW/d, and significantly decreased hepatic cytochrome b5, cytochrome P450, and MDA levels also were noted in the developing fetuses and neonates whose mothers were administered 500 mg/kg BW/d. The dose of 100 mg/kg BW/d by gavage had no effect on hepatic SH, GST, cytochrome b5, cytochrome P450, or MDA levels. No other treatment-related effects were reported.

3.5. Animal efficacy studies showing no adverse effects of *C. vulgaris*

Several efficacy studies reported no side effects *C. vulgaris* in mice, rats, or rabbits administered diets supplemented with the algae at concentrations up to 13.8% for durations up to 9 wk (Table 6; An et al., 2008; Janczyk et al., 2007; Konish et al., 2002; Lee et al., 2008; Mizoguchi et al., 2010; Morris et al., 2007; Sano et al., 1988; Sano and Tanaka, 1987). Reported health benefits include hypoglycemic (Lee et al., 2009), hypocholesteolemic (Lee et al., 2008), and antifatigue effects (An et al., 2008; Mizoguchi et al., 2010), and immune-potentiating activities (Morris et al., 2007) as well as nutritional values (Janczyk et al., 2007). No toxicologically significant adverse effects were noted following biochemical analyses.

Lee et al. (2009) examined the effect of *Chlorella vulgaris* on glucose metabolism in rats fed a high fat diet. Sixty 6-week-old male Wistar rats were divided into two groups: normal diet group (N group) and high fat diet group (H group). Then the rats in each group were further divided into three subgroups and fed either *Chlorella*-free diets or diets with 5% (C5) or 10% (C10) (wt/wt) *Chlorella* for 9 wk. Body weight gain, feed intake, and feed efficiency ratio did not differ among the subgroups with the normal and high fat diet groups. The fasting glucose and insulin concentrations, as well as insulin resistance (homeostasis model assessment), decreased in a dose response manner in high fat diet groups. In the high fat groups, 10% *Chlorella* intake was more effective in blood glucose regulation than 5% *Chlorella* intake in rats fed a high fat diet (fasting blood glucose: control, 194.2^{a} vs. HC5, 183.5^{ab} vs. HC10, 171.4^{b} mg/dl, P; fasting insulin: control, 1.80^{a} vs. HC5, 1.65^{b} vs. HC10, 1.29^{c} ug/L; HOMA-IR: control, 15.8^{a} vs. HC5, 13.5^{b} vs. HC10, 9.8^{c}; values with different letters differ, $p<0.05$). In rats fed normal fat diets, fasting blood glucose did not significantly differ among the groups, but the *Chlorella* group had lower plasma insulin and HOMA values (fasting insulin: control, 1.33^{a} vs. HC5, 1.21^{b} vs. HC10, 0.99^{c} ug/l; HOMA-IR: control, 9.0^{a} vs. HC5, 7.1^{b} vs. HC10, 5.9^{b}). Authors concluded that *Chlorella* intake may prevent insulin resistance in Wistar rats fed a high fat diet. No side effects were reported.

An et al. (2006) investigated the effect of *C. vulgaris* on a forced swimming test and blood biochemical characteristics related to fatigue, blood urea nitrogen (BUN), creatine kinase (CK), lactic dehydrogenase (LDH), glucose, and total protein (TP). C. *vulgaris* extract (50, 100, or 150 mg/kg/d) was orally administered to mice. After 7 d, the immobility time was decreased in the 100 and 150 mg/kg *C. vulgaris* -treated groups (179 +/- 8.3 and 175 +/- 2.1 s) in comparison with the control group (223 +/- 5.4 s). There were no significant differences in blood glucose, total protein, CK, or LDH concentrations. The BUN concentrations did not show dose-related responses. No

adverse effects of C. *vulgaris* were reported. Kim et al. (2010) also reported that mice receiving *Chlorella vulgaris* (heat-treated) for 14 d reduced the immobility time in a forced swimming test as compared to the control (control, 123 vs. *Chlorella* group, 92.2 s, $p < 0.01$).

Mizoguchi et al. (2010) also reported that *Chlorella* intake attenuated swimming stress in mice: the global expression profile of muscle from the *Chlorella vulgaris* mice was similar to that of non-swimming mice rather than to that of control swimming mice. No adverse effects of *Chlorella vulgaris* were reported.

Morris et al. (2007) reported the immunopotentiating activity of *Chlorella* protein hydrolysate in both innate and specific immune responses of undernourished Balb/c mice after a 3-d fasting period. Protein hydrolysate from *Chlorella vulgaris* was prepared by hydrolysis of ethanol-extracted cell biomass with pancreatin (20 AU/g) at pH 7.5 and 45 °C for 4 h. The treatment with protein hydrolysate from *Chlorella vulgaris* (500 mg/kg) for 8 d provided benefits in terms of hemopoiesis, as judged by the recovery of bone marrow cellularity and the leukocyte counts in peripheral blood, particularly the lymphocyte pool, which increased up to 128% compared to control animals.

Sano and Tanaka (1987) also reported the anti-lipidemic action and anti-atherosclerotic action of dried, powdered *Chlorella vulgaris* in male Japanese White rabbits fed a high-cholesterol diet containing 1% powdered *Chlorella vulgaris* for 10 wk. No adverse effects of *Chlorella vulgaris* were reported. The enhancement of cholesterol catabolism through up-regulation of hepatic CYP7A1 expression contributes to the hypocholesterolemic effect (Shibata et al., 2007).

The glycoprotein fraction is known as an active component of *Chlorella vulgaris* which is responsible for immunopotentiating activity (Tanaka et al., 1998). Sano et al. (1988) reported that glycolipid (GL) and phospholipid (PL) fractions obtained from *Chlorella vulgaris* inhibited the increase in concentration of serum lipids in cholesterol-fed rats. Fecal excretion of steroids (mostly of cholesterol and deoxycholic and lithocholic acids) were increased by feeding GL and PL fractions. No adverse effects of *Chlorella vulgaris* were reported.

Table 6. Animal efficacy studies reporting no adverse effects of *Chlorella vulgaris*

Species	Dose	Length	Measurements	Ref
Rat, Wistar	0, 5, or 10% in normal diet or high fat diet	9 wk	BW gain, feed intake, FER, fasting glucose and insulin conc., HOMA-IR	Lee et al. 2009
Mice	50, 100, or 150 mg/kg/d	7 d	Blood metabolites related to fatigue, BUN, CK, LDH, TP, and glucose	An et al., 2006
Rat, Wistar	Lipophilic extract of *C. vulgaris*, 0 or 7,500 mg/kg/d (cholesterol-enriched feed, 0 or 5%)	1 wk	Serum TC and PL levels and fecal excretion of steroids	Sano et al., 1988
Mice, undernourished Balb/c	Protein hydrolysate from *C. vulgaris;* 500 mg/kg BW/d	8 d	Hemopoiesis	Morris et al. 2007
Rabbit, Japanese white	0 or 363 mg/kg/d (a cholesterol enriched diet, 0 or 1%)	10 wk	Serum TC, β-lipoprotein concentrations, and atherosclerotic development	Sano and Tanaka, 1987
Rat	*C. vulgaris,* up to 20% in the diet w/ 0.9% DHA, 1% cholesterol, 0.5% cholate; up to 20 g/d	4 wk	Blood lipid profiles	Konish et al., 2002

ALT = alanine transaminase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; F = female; PL = phospholipid; T = treatment; TC = total cholesterol; TG = triglycerides; FER= feed efficiency ratio; BUN=blood urea nitrogen; CK=creatine kinase, LDH=lactic dehydrogenase, TP=total protein

3.6. Toxicity or animal efficacy studies of unspecified or other *Chlorella* species

Studies on other strains of *Chlorella* sp. also reported no signs of clinical toxicity; there were no effects on final body weight, BW gain, food intake, or food efficiency ratios, hematological or histological characteristics or liver weights in rats, mice, hamsters, broiler chicks, laying hens, or piglets administered diets supplemented with *Chlorella* species at concentrations up to 15% for durations between 1 and 21 wk (Table 7; Bedirli et al., 2009; Cherng and Shih, 2005; Chovancikova and Simek, 2001; Day et al., 2009; Herrero et al., 1993; Okuda et al., 1975; Sansawa et al., 2006; Yap et al., 1982).

Day et al. (2009) investigated the safety of *Chlorella protothecoides* in a 28-d study. Sprague–Dawley rats were administered 0 (control), 2.5, 5.0, or 10% (corresponding to 0, 1,794, 3,667, and 7,557 mg/kg BW for males and 0, 1,867, 3,918, and 8,068 mg/kg BW for females) of their diet for 28 d using an FDA Redbook protocol. Although statistically significant alterations were noted in several characteristics between males and females, these changes were deemed to be of no toxicological significance. It is due to the following: 1) lack of dose–response relationships, 2) responses occurred in only one sex, and 3) lack of any supporting gross or microscopic alterations. The authors determined the NOAEL levels to be 10% in the diet (7,557 and 8,068 mg/kg BW/d for males and females, respectively), the highest doses tested.

Lipstein and Hurwitz (1980) found no abnormalities in reproductive performance in laying hen fed 15% *Chlorella* for 8 wk. Broiler chicks fed 6 or 15% *Chlorella* for 8 wk had normal growth performance.

Okuda et al. (1975) reported hypocholesterolemic effects of *Chlorella* (unspecified strain) in mice. In this study, mice were fed a hypercholesterolemic diet containing 2% cholesterol with or without 10% *Chlorella* for 7 d. No adverse effects of *Chlorella* were reported. Kanomori et al. (1983) also reported hypocholesterolimc effects of *Chlorella* in rats.

Yorkshire piglets consumed *Chlorella* (unspecified strain) at a level of 13.81% from d 4 to 15 or d 8 to 26 of age (equivalent to approximately 600 and 500 mg/kg BW/day, respectively) showed no adverse effects (Yap et al., 1982).

Furthermore, no biologically significant adverse effects on growth or food intake were reported following the provision of diets containing 7.2% *C. pyrenoidosa* (approximately 8,640 mg/kg BW/d) to Wistar rats or hamsters for 8 wk (Cherng and Shih, 2005) and diets containing 1% *C. pyrenoidosa* (approximately 8,640 mg/kg BW/day) to mice (Chovancikova and Simek, 2001).

Supplementation of *Chlorella regularis* (10-20% in diet) for 21 wk significantly reduced blood pressure by 8.2% (256 vs. 235 mm Hg) in stroke-prone spontaneously hypertensive rats (Sansawa et al., 2006). Histopathological examination revealed cerebral vascular accidents in the brains of the control group, but those of *Chlorella*

groups showed apparently low incidence (1/5) compared to the control group (5/5). Adenosine and glycine (Murakami et al., 1980) have been identified as active components responsible for modulating blood pressure.

Konish et al. (2002) found that atherogenic diets containing 1% cholesterol and 0.5% cholate did not increase serum cholesterol concentrations in rats when fed 5-20% *Chlorella vulgaris* and 0.9% DHA in the diet.

Table 7. Animal safety/efficacy studies reporting no side effects of unspecified or other species of *Chlorella*

Species	Chlorella strain; Dose	Length	Measurements	Ref
Rats, Sprague–Dawley	*Chlorella protothecoides;* 2.5, 5, or 10% in diet	28 d	Traditional subacute toxicity study characteristics	Day et al., 2009
Broiler chicks	Unspecified; 6 or 15%	8 wk	Growth, feed utilization, abdominal fat, liver lipids	Lipstein and Hurwitz, 1980
Laying hen	Unspecified; 6 or 15%	2 mo	Body wt and reproductive performance (egg production, egg wt, egg lipid composition)	Lipstein and Hurwitz, 1980
Mice	Unspecified; 0 or 10% (with 2% cholesterol diet)	7 d	Liver lipid profile	Okuda et al., 1975
Piglet, Yorkshire	Unspecified; 13.81%	11 or 18 d	Body weight	Yap et al., 1982
Rat, Wistar	Unspecified; 0 or 50 mg/kg BW/d gavage	10 d	Plasma endotoxin conc., evidence of bacterial translocation, MLNs and liver oxidative stress, and histology	Bedirli et al., 2009
Rat, Wistar	*C. pyrenoidosa*; 0.9, 1.8, or 7.2% with 5% cholesterol diet	8 wk	Serum lipid profiles	Cherng and Shih, 2005
Hamster	*C. pyrenoidosa*; 0.9, 1.8, or 7.2% with 1% cholesterol diet	8 wk	Serum lipid profiles	Cherng and Shih, 2005
Mouse, CD1	*C. pyrenoidosa*; Standard feed or high fat feed, 0 or 1%	10 wk	Serum lipid profiles	Chovancikova and Simek, 2001
Rat, Wistar albino	*C. stigmatophora*; at a protein conc. of 12%	4 wk	Food intake, PER, FCE	Herrero et al., 1993
Rat, hypertensive	*C. regularis;* 10% in diet	21 wk	Blood pressure, blood lipid profiles, histopathological exam.	Sansawa et al., 2006

PER=protein efficiency ratio; FCE=food conversion efficiency; PL= phospholipid; MLNs=mesenteric lymph nodes

3.7. Mutagenicity and genotoxicity studies

No mutagenicity or genotoxicity studies were identified in the literature. However, several studies reported antigenotoxic effects of *Chlorella vulgaris*. For example, *Chlorella vulgaris* exhibited protective effects against hydrogen peroxide-induced DNA damage and telomere shortening of human fibroblasts derived from different aged individuals (Makpol et al., 2009). Also, Sarma et al. (1993) reported that *Chlorella vulgaris* had protective effects against the gamma-ray induced chromosomal damage (a micronucleus test) in whole-body irradiated mice. A significant radioprotective effect was observed in both acute and chronic pretreatments, but only at doses above 400 mg/kg BW. However, in mice that received E-25 (500 mg/kg) three times a day for 28 d, there was no protective effect, and a significant loss in their BW was observed.

A chlorophyll sample prepared from *Chlorella vulgaris* inhibited the mutagenicity of 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole, a direct-acting mutagen, in Salmonella (Negeshi et al., 1997).

3.8. Carcinogenicity studies

No traditional carcinogenicity studies were identified in the literature. However, there are reports that *Chlorella vulgaris* is anticarcinogenic in mice and rats (Azamai et al., 2009; Hasegawa et al., 2000; Justo et al., 2001; Konishi et al., 1985, 1996; Morimoto et al., 1995; Ramos et al., 2010; Tanaka et al., 1984, 1990; Wang et al., 1979, 1980, 2010). For example, *C. vulgaris* dried powder or its acetone extract administered in the diet for up to 57 d at levels of up to 15 g/kg BW/d did not promote the growth of subcutaneously inoculated 3- methylcholanthrene-induced tumor cells in CDFI mice (Tanaka *et al.,* 1990). Additionally, the authors reported that dietary administration of *C. vulgaris* dried powder resulted in no serious side effects including decreases in BW or other wasting syndromes. Bone marrow colony formation was significantly increased and survival was prolonged in male BALB/c mice inoculated with Erlich ascites tumor following the administration of *C. vulgaris* extract by gavage for 5 d at doses of 50, 100, or 200 mg/kg BW/d compared to placebo tumor-bearing mice (Justo et al., 2001).

In a cell proliferation assay, *C. vulgaris* extract inhibited human lung cancer H1299, A549, and H1437 cells in a dose-dependent manner. In addition, the treatment with extracts of *C. vulgaris* effectively reduced the migration of tumor cells (Wang et al., 2010).

Azamai et al. (2009) reported that *Chlorella vulgaris* triggered apoptosis in hepatocarcinogenesis-induced rats. Male Wistar rats were divided into eight groups: control group (normal diet), CDE group (choline-deficient diet supplemented with ethionine in drinking water to induce hepatocarcinogenesis), *Chlorella vulgaris* groups with three different doses (50, 150, and 300 mg/kg BW/d), and a choline-deficient diet group treated with different doses of *Chlorella vulgaris* (50, 150, and 300 mg/kg BW/d). *Chlorella vulgaris,* at increasing doses, decreased the expression of anti-apoptotic

protein, Bcl-2, but increased the expression of pro-apoptotic protein, caspase 8, in choline-deficient diet rats, which was correlated with decreased hepatocyte proliferation and increased apoptosis as determined by bromodeoxy-uridine (BrdU) labeling and terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) assay, respectively. The results showed that *Chlorella vulgaris* had chemopreventive effects by inducing apoptosis via decreasing the expression of Bcl-2 and increasing the expression of caspase 8 in hepatocarcinogenesis-induced rats. Wang et al. (1979, 1980) also reported a protective role of *C. vulgaris* against ethionine-induced hepatotoxicity. Monogalactosyl diacylglycerols (Morimoto et al. 1995), glycoprotein (Hiroshi et al., 1983; Noda et al. 1996), and sterols (Yasukawa et al., 1996) have been identified as anti-tumor agents present in *Chlorella vulgaris.*

3.9. Antioxidant effects of *Chlorella vulgaris*

Chlorella vulgaris extract had strong antioxidant activities such as radical scavenging, ferric reducing power and attenuating oxidative stress (Azizzat et al., 2010; Bedirli et al., 2009; Estevez et al., 2001; Lee at al., 2003; Miranda et al., 2001; Shibata et al., 2003; Vijayavel et al., 2008; Wang et al., 2010; Wu et al., 2005). Rodriguez-Garcia et al. (2008) reported that the activity of *C. vulgaris* extract was higher than those obtained for the synthetics, BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene).

3.10. Antitoxic effects against metal-induced toxicity

Chlorella vulgaris extracts are known to have strong chelating abilities to metals (Cd, Mn, Cr, Ni, Zn, Fe, and Cu) to attenuate metal-induced damages (Allnut et al., 1987;El-naggar et al., 1998; Huang et al., 2009; Hwang et al., 2006; Jang et al., 2008; Kim et al., 2009; López-Suárez et al., 2000; Mallick et al., 2004; Queiroz et al., 2003, 2008a; Shim et al., 2008, 2009; Son et al., 2009; Wang et al., 2010; Yang et al., 2008). For example, Shim et al. (2008) reported protective effects of *Chlorella vulgaris* against cadmium (Cd)-induced liver toxicity in male Sprague-Dawley rats (5 wk of age, weighing 90–110 g). Forty rats were fed one control and three groups treated with 10 ppm Cd: one Cd without *Chlorella* (Cd control), one Cd with 5% *Chlorella* (Cd-5% C. vulgaris), and one Cd with 10% *Chlorella* (Cd-5% C. vulgaris) groups for 8 wk. Body weight gain and relative liver weight were significantly lower in the Cd-0C group than in Cd-5C and Cd-10C groups. Rats in the Cd-0C group had significantly higher hepatic concentrations of Cd and metallothioneins (MTs) than in the Cd-5C or Cd-10C group. Morphologically, a higher level of congestion and vacuolation was observed in the livers of the Cd-0C group compared to those of the Cd-5C and Cd-10C groups. Other researchers reported protective effects of *Chlorella vulgaris* against cadmium (Cd)-induced toxicity in rats and mice (Naggar and El-Sheekh, 1998; Huang et al., 2009; Kim et al., 2001; Shim et al., 2009; Yang et al., 2008). However, Kim et al. (2009) reported that *Chlorella vulgaris* supplementation (up to 10% in the diet) did not improve Cd detoxification from the body of growing rats.

Also, administration of *Chlorella vulgaris* extract (50 mg/kg/d for 8 d) protected the host from Pb (1300 ppm)-induced damages (Queiroz et al., 2003, 2008). The group that

was simultaneously exposed to *Chlorella vulgaris* extract /Pb had a dramatic reduction of 66.03% in blood Pb concentrations, when compared to Pb-exposed non-treated control (Queiroz et al., 2003). On the other hand, *Chlorella vulgaris* extract treatment following Pb exposure produced a much less effective chelating effect: *Chlorella vulgaris* extract treatments for 3 - 10 d, starting 24 h following Pb exposure, resulted in a reduction in blood Pb concentrations of 13.5-17% compared to Pb-exposed non-treated controls. The significantly better response observed with the simultaneous *Chlorella vulgaris* extract/Pb administration indicates that the immunomodulation effect of *Chlorella vulgaris* extract plays an important role in the ability of this algae to reduce blood Pb concentrations.

Tanaka et al. (2001) reported that a protein moiety (glycoprotein) present in *Chlorella vulgaris* was essential for CVS to exhibit immunopotentiating activity. In the study of Jang et al. (2008), the administration of *Chlorella vulgaris* showed antitoxic effects against Pb-induced oxidative stress especially associated with the liver fibrosis. Six week old Sprague-Dawley male rats (n=10/group) were randomly divided into control group (Control; treated with drinking water) and Pb-*Chlorella* groups which were further divided into Pb, 200 ppm Pb acetate control, Pb+2% *Chlorella*, Pb+5% *Chlorella* and Pb+10% *Chlorella* in diet. During the 4 wk test period, superoxide dismutase activity was significantly decreased in the Pb-positive control group compared to Pb-negative control but was significantly increased in Pb+C2, Pb+C5, and Pb+C10 (128.9, 165.8 and 167.4% respectively) compared to the Pb-positive control group ($P<0.05$). Glutathione peroxidase and glutathione reductase activity showed a similar pattern.

3.11. Antitoxic effects of *Chlorella vulgaris* against infection

Chlorella treatment was effective for the prophylaxis of post-stress myelossupression such as in vivo challenge with *Listeria monocytogenes* or *E. coli* (Konish et al., 1990; Tanaka et al., 1986). Several studies demonstrated that *Chlorella vulgaris* extract (50 mg/kg BW/d for 5 -10 d) produced a significant increase in the resistance of the animals infected with *L. monocytogenes* (Dantas and Queiroz, 2006; Queiroz et al., 2003, 2008b) through augmentation of helper T cell type 1 (Thl) responses producing gamma-interferon (gammaIFNl; Hasegawa et al., 1999). Also, oral administration of hot water extract of *Chlorella vulgaris* enhanced resistance to *Listeria monocytogenes* through augmentation of *Listeria*-specific cell-mediated immunity in normal mice and mice with murine acquired immunodeficiency syndrome (MAIDS) caused by murine leukemia virus *(MuLV) LP-BM5* (Hasegawa et al., 1995, 1997) as *Chlorella vulgaris* extract improved the deteriorated immune response to *L. monocytogenes* (Hasegawa et al., 1995, 1997). Glycoprotein present in *Chlorella vulgaris* contributes to immunopotentiating effects of *Chlorella vulgaris* (Tanaka et al., 1998).

Singh et al. (1995) reported protective effects of *Chlorella vulgaris* (500 mg/kg) against sublethal gamma ray-induced damage. $LD_{50/30}$ for Chlorella pre- and post-treated mice were 8.66 and 9.0 Gy, respectively, compared to the control value of 7.8 Gy. The dose reduction factor (DRF) was 1.11 and 1.15 for pre-treated and post-treated mice

respectively. Hoshikawa et al. (1993) also reported a protective role of *Chlorella vulgaris* against X-irradiation damage in mice.

4. Human clinical studies

Human clinical studies reported no adverse effects related to the consumption of *Chlorella*. The consumption of *Chlorella vulgaris* up to 20 g/d did not show adverse effects (Table 8; Lee et al., 2010; Tanana et al., 2010). Also, other species of *Chlorella* was well-tolerated at concentrations up to 100 g/d (Powell et al., 1961).

Lee et al. (2010) investigated if *Chlorella* supplementation to smokers was protective against oxidative damage in 52 smokers given 6.3 g of *Chlorella vulgaris* for 6 wk. *Chlorella* supplementation increased plasma vitamin C (44.4%), alpha-tocopherol (15.7%), and erythrocyte catalase and superoxide dismutase activities. The consumption of *Chlorella* had no adverse effects on lymphocyte DNA damage (a marker of oxidative stress), as measured by the comet assay. The authors concluded that supplementation of *Chlorella vulgaris* improved plasma antioxidant nutrient status erythrocyte antioxidant enzyme activities in male smokers. No side effects were reported.

Tanaka et al. (2002) reported no adverse effects of *Chlorella vulgaris* from a human clinical trial in which *Chlorella vulgaris* (up to 20 g/d), enriched with DHA (150 mg/d), was administered for 9 wk in 6 middle-aged participants. For the first 2 wk, 4 g/d was supplemented with DHA (150 mg). At 2-wk intervals, the dose was increased progressively to 8 and 12 g, after which 20 g/d was administered for the last 3 wk. The *Chlorella vulgaris* treatments did not change the other blood characteristics, and no adverse effects, except positive changes in total cholesterol concentrations, were observed in these experiments.

Table 8. Human safety studies reporting no adverse effects of *Chlorella vulgaris*

Subjects	Dose	Length	Measurements	Ref
52 healthy smoking males, 20-65 yr	*C. vulgaris;* 6.3 g/d	6 wk	Lymphocyte DNA damage (a marker of oxidative stress), plasma antioxidant nutrient status	Lee et al., 2010
6 healthy subjects	*C. vulgaris;* up to 20 g/d, w/ 150 mg DHA	9 wk	Blood lipid profiles	Tanaka et al., 2002

The studies investigating the short- and long-term effects of other species of *Chlorella* (unspecified strain and *C. pyrenoidosa*) also reported no adverse effects when used up to 100 g/d (Table 9; Dam et al.,1965; Halperin et al., 2003; Inoue et al., 1995; Merchant and Andre, 2001; Merchant et al., 1999, 2000, 2002; Mizoguchi et al., 2008; Okuda et al., 1975; Powell et al., 1961). The only adverse effects reported were symptoms of gastrointestinal upset, nausea, and fever in 20 subjects with malignant glioma during the first week of treatment with 20 g of *C. pyrenoidosa* and 150 mL of a liquid *C. pyrenoidosa* extract in a 2-yr study (Merchant et al., 1990). These gastrointestinal effects were transient and disappeared starting the second week of the study as subjects adapted to a high consumption of *Chlorella*.

Overall, findings from human clinical studies support a long history of safe human use of Chlorella. No animal or human clinical studies reported adverse effects related to consumption of *Chlorella vulgaris.*

Table 9. Human safety studies reporting no adverse effects of unspecified or other species of *Chlorella*

Subjects	Dose	Length	Measurements	Ref
16 hypercholesterolemic subjects	Unspecified; 5 g/d	3 mo	Serum cholesterol concentration.	Okuda et al., 1975
23 hypertensive subjects, mean age of 53.8 yr, 11 F, 12 M	Unspecified; 1.5 g/d	6 mo	Arrhythmia and myocardial ischemia symptoms, serum lipid profiles and uric acid and fasting blood glucose concentrations	Inoue et al., 1995
5 healthy males, 18- 23 yr	*C. pyrenoidosa;* 100 g/d	26 d	Gastrointestinal tolerance of algae, hematology, urinalysis, and liver function tests	Powell et al., 1961
43 pregnant women 23-40 yr	*C. pyrenoidosa;* 6 g/d	6 mo from gestational wk 12-16 to delivery	Dioxin total toxic equivalents in blood, breast milk, and adipose tissues	Nakano et al., 2005
17 healthy males, mean age 34.3 yr and 17 males w/ high-risk of lifestyle-related disease, mean age 59.2 yr	*C. pyrenoidosa;* 7.64 g/d	12 wk	Body fat, serum lipid profiles, gene expression profiles	Mizoguchi et al., 2008
24 subjects w/ mild/moderate hypertension	*C. pyrenoidosa;* 10 g/d tablet and 100 mL extract	1-2 mo	Blood pressure, quality of life	Merchant et al., 2002
37 subjects w/ fibromyalgia syndrome, 36 F, 1 M	*C. pyrenoidosa;* 10 g/d tablet and 100 mL extract	3 mo	Physical exam, and hematological and urinalysis characteristics	Merchant and Andre, 2001
Sex not specified	*C. pyrenoidosa;* 20 g/d and 150 mL (extract)	up to 2 y	Hematology, immunological characteristics (circulating concentrations of monocytes, leukocytes, and granulocytes, proportion of lymphocytes bearing specific T-cell and natural killer cell markers)	Merchant et al., 1990
124 healthy subjects, 50 to 89 yr; 29 M, 95 F	*C. pyrenoidosa;* 200 or 400 mg/d	28 d	Immunological characteristics (antibody response to influenza vaccine), liver enzymes, complete blood counts	Halperin et al., 2003
5 healthy subjects, 24-35 yr; 4 M, I F	*C. pyrenoidosa;* 54.2 and 90.3 g/d (ethanolic extract)	10 d	Nitrogen balance, apparent digestibility of nutrients	Dam et al., 1965
6 health subjects, 18-32 yr; 3 M, 3 F	*C. pyrenoidosa;* 57.3 g/d	5 d	Nitrogen balance, body weight, calorie intake	Lee et al., 1967

5. Algal cyanobacterial toxin and pheophorbide analysis

The Japanese Public Health Ministry recommended that the level of pheophorbide, a breakdown product of chlorophyll a reported to cause photosensitive dermatitis in humans, in algae preparations be restricted to less than 1.2 mg/g (Becker, 1994). The analysis of 2 non-consecutive lots of RFI's *Chlorella* demonstrated that the level of pheophorbide-a in RFI's *Chlorella* (not detected to 0.0334 mg/g) is below the limit established by the Japanese Public Health Ministry. The results of the studies conducted in humans with the oral administration of various species of *Chlorella* do not indicate any potential for toxicity or cause for concern resulting from the consumption of RFI's *Chlorella* under the conditions of intended use.

None of the algal or cyanobacterial toxins that have been identified in the published literature or mentioned in international food regulations, such as microcystins, nodularin , anatoxin-a, cylindrospermopsins, and β-methylamino-L-alanine, were detected in RFI's *Chlorella.*

6. Allergy

No case of allergy was identified in the scientific literature following consumption of *Chlorella*. Proteins of *C. vulgaris*, *C. saccharophila*, or *C. hornosphera* do not have significant allergenic potential, even in atopic individuals (Tiberg *et al*., 1990a, 1990b, 1995).

IV. Conclusions

This GRAS determination for *Chlorella vulgaris* is based upon scientific procedures and through experience based on common use in foods and beverages. *Chlorella vulgaris* has a long history of safe use in foods. *Chlorella vulgaris* is of natural biological origin and has been widely consumed for its nutrient properties, without known detrimental effect. There is abundant literature describing the composition of *Chlorella vulgaris*. Also, numerous human and animal studies examined the health benefits of *Chlorella vulgaris* and other *Chlorella* species. There are no reports of safety concerns in any of the studies. RFI utilizes a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications.

The information/data provided by RFI in this report and supplemented by the publicly available literature/toxicity data on *Chlorella vulgaris* provide a sufficient basis for an assessment of the safety of *Chlorella vulgaris* for the proposed use as an ingredient in foods and beverages, when prepared according to appropriate specifications and used according to GMP.

Key findings are summarized here:

1. *Chlorella vulgaris* is well characterized and free from chemical and microbial contamination.
2. *Chlorella vulgaris* has a long history of safe use in foods.
3. Manufacturing processes of *Chlorella vulgaris* have been safely used for many years in the food industry.
4. The safety and nutritional benefits of *Chlorella vulgaris* are well established by human clinical trials and animal studies. There are no indications of significant adverse effects related to *Chlorella vulgaris* consumption in the publicly available literature.
5. The level of pheophorbides, a breakdown product of chlorophyll, in RFI's algal *Chlorella* is considerably lower than the limit established by the Japanese Public Health Ministry.

Therefore, not only is the proposed use of *Chlorella vulgaris* in foods and beverages safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also GRAS.

V. Discussion of information inconsistent with GRAS determination

We are not aware of information that would be inconsistent with a finding that the proposed use of *Chlorella vulgaris* preparations in foods and beverages, meeting appropriate specifications and used according to cGMP, is GRAS.

VI. Availability of information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

RFI, Inc.
300 Corporate Drive, Suite 14
Blauvelt, NY 10913
USA

Should the FDA have any questions or additional information requests regarding this notification, RFI, Inc. will supply these data and information.

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Appendix: Material Safety Data Sheet

Prepared by: RFI Ingredients
300 Corporate Drive Suite 14
Blauvelt, NY 10913
For Information: (845) 358 - 8600

MATERIAL IDENTIFICATION AND INFORMATION:

Product Identification:	Chlorella Powder BCW RFI-GC130015
Synonym:	N/A
CAS:	N/A
DOT Classification:	Non-hazardous

COMPONENTS: CHEMICAL AND COMMON NAMES	% (W/W)	**OSHA PEL**	TLV
Chlorella Powder	100%	N/A	N/A

PHYSICAL AND CHEMICAL CHARACTERISTICS:

APPEARANCE & ODOR	SPECIFIC GRAVITY (@20/20^{O}C)	SOLUBILITY IN WATER	MELTING POINT	BOILING POINT	VAPOR PRESSURE	EVAPORA-TION RATE	VAPOR DENSITY (AIR =1)
Fine green Powder	N/A	Dispersible	Not Known	N/A	N/A	N/A	N/A

FIRE AND EXPLOSION HAZARD DATA:

Flash point	EXPLOSION HAZARDS	SPECIAL FIRE FIGHTING PROCEDURES	INCOMPATIBILITY (MATERIALS TO AVOID)	HAZARDOUS COMBUSTION PRODUCTS	EXTINGUISH MEDIA	HAZARDOUS POLYMERIZATION
N/A	Not known	Use standard procedures and preferred extinguishing media above.	Oxidizing materials can cause a reaction	Burning generates CO, CO_2, irritating smoke	Water Fog CO_2 Foam Dry Chemical	Will not occur

SPILL & LEAK PROCEDURES:

If Released or spilled	**WASTE DISPOSAL METHOD**
Soak up spills with absorbent media; collect in containers for disposal. Avoid open flame or other sources of ignition.	Treat, store, and dispose in accordance with Federal, State, and Local regulations. Material is biodegradable and maybe treated via sanitary waste treatment plant or approved sanitary landfill.

HEALTH HAZARD DATA:

PRIMARY ROUTES OF ENTRY	CARCINOGEN LISTED IN	HEALTH HAZARDS	MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE
Absorption Ingestion; inhalation	Not Listed.	This mixture has not been tested as a whole. The mixture includes, however, ingredients at concentrations greater than or equal 1% which, undiluted, could present the following hazards to health: SKIN/EYES: May irritate skin & eyes INGESTION: No adverse effects	Not known for normal conditions of use.

		anticipated INHALATION: No adverse effects anticipated	

Emergency first aid procedures- seek medical assistance for further treatment, observations, and support if necessary:

EYE CONTACT	**SKIN CONTACT**	**INHALATION**	**INGESTION**
Immediately flush with large amounts of water for at least 15 minutes.	N/A	Not Known	Not Known

CONTROL AND PROTECTIVE MEASURES:

RESPIRATORY PROTECTION	HANDLING AND STORAGE PRECAUTIONS	OTHER PROTECTIVE CLOTHING AND EQUIPMENT	HYGIENIC WORK PRACTICES	ENGINEERING CONTROLS
Dust mask.	Keep containers closed.	Protective gloves.	Ware standard work gloves, standard process and lab ware and hats	Provide adequate ventilation. Eyewash and safety showers should be easily accessible.

SECTION 313 SUPPLIER NOTIFICATION:

This product contains the following toxic chemicals subject to the reporting requirements of Section 313 of the Emergency Planning and Community Right To Know Act of 1986 and of 40 CFR 372.

Chemical	CAS#	% by Weight
N/A		

The information contained herein is provided in good faith and is believed to be correct as of the date hereof. However, RFI Ingredients makes no representation as to the comprehensiveness or accuracy of the information. It is expected that individuals receiving the information will exercise their independent judgment in determining its appropriateness for a particular purpose.

Material Safety Data Sheet
Prepared by: RFI Ingredients
300 Corporate Drive Suite 14
Blauvelt, NY 10913
For Information: (845) 358 - 8600

I. MATERIAL IDENTIFICATION AND INFORMATION:

Product Identification: Chlorella Powder – BCW Organic

Synonym: ***N/A***

CAS: N/A

DOT Classification: *Non-hazardous*

COMPONENTS: CHEMICAL AND COMMON NAMES	% (W/W)	A. OSHA PEL	TLV
Chlorella Powder – BCW Organic	100%	N/A	N/A

II. PHYSICAL AND CHEMICAL CHARACTERISTICS:

APPEARANCE & ODOR	SPECIFIC GRAVITY (@20/20^{O}C)	SOLUBI-LITY IN WATER	MELTING POINT	BOILING POINT	VAPOR PRESSURE	EVAPORA-TION RATE	VAPOR DEN-SITY (AIR =1)
Fine green Powder	N/A	Dispersible	Not Known	N/A	N/A	N/A	N/A

III. FIRE AND EXPLOSION HAZARD DATA:

Flash point	EXPLO-SION HAZARDS	SPECIAL FIRE FIGHTING PROCE-DURES	INCOMPATIBILITY (MATERIALS TO AVOID)	HAZARDOUS COMBUSTION PRODUCTS	EXTINGUISH MEDIA	HAZARDOUS POLYMERIZA-TION
N/A	None Known	Use standard procedures and preferred extinguishing media above.	Oxidizing materials can cause a reaction	Burning generates CO, CO_2, irritating smoke	Water Fog CO_2 Foam Dry Chemical	Will not occur

IV. SPILL & LEAK PROCEDURES:

If Released or spilled	**WASTE DISPOSAL METHOD**
Soak up spills with absorbent media; collect in containers for disposal. Avoid open flame or other sources of ignition.	Treat, store, and dispose in accordance with Federal, State, and Local regulations. Material is biodegradable and maybe treated via sanitary waste treatment plant or approved sanitary landfill.

V. HEALTH HAZARD DATA:

PRIMARY ROUTES OF ENTRY	CARCINOGEN LISTED IN	HEALTH HAZARDS	MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE
Absorption Ingestion; inhalation	Not Listed.	This mixture has not been tested as a whole. The mixture includes, however, ingredients at concentrations greater than or equal 1% which, undiluted, could present the following hazards to health: SKIN/EYES: May irritate skin & eyes INGESTION: No adverse effects anticipated INHALATION: No adverse effects anticipated	Not known for normal conditions of use.

EMERGENCY FIRST AID PROCEDURES- SEEK MEDICAL ASSISTANCE FOR FURTH TREATMENT, OBSERVATIONS, AND SUPPORT IF NECESSARY:

EYE CONTACT	SKIN CONTACT	INHA-LATION	INGESTION
Immediately flush with large amounts of water for at least 15 minutes.	N/A	Not known	Not known

VI. CONTROL AND PROTECTIVE MEASURES:

RESPIRATORY PROTECTION	HANDLING AND STORAGE PRECAU-TIONS	OTHER PROTECTIVE CLOTHING AND EQUIPMENT	HYGIENIC WORK PRACTICES	ENGINEERING CONTROLS

Dust mask.	Keep containers closed.	Protective gloves.	Ware standard work gloves, standard process and lab ware and hats	Provide adequate ventilation. Eyewash and safety showers should be easily accessible.

VII. SECTION 313 SUPPLIER NOTIFICATION:

This product contains the following toxic chemicals subject to the reporting requirements of Section 313 of the Emergency Planning and Community Right To Know Act of 1986 and of 40 CFR 372.

Chemical	CAS#	% by Weight
N/A		

The information contained herein is provided in good faith and is believed to be correct as of the date hereof. However, RFI Ingredients makes no representation as to the comprehensiveness or accuracy of the information. It is expected that individuals receiving the information will exercise their independent judgment in determining its appropriateness for a particular purpose.

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SUBMISSION END

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